1785-Plat
Surface-Free Single-Molecule Force Spectroscopy
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Mechanical forces play key roles throughout biology. Most existing methods for probing forces in/between molecules and cells, such as the AFM, optical tweezers, magnetic tweezers, traction force microscopy, etc., require molecules to be tethered to the surfaces of cover glasses or beads. However, surface tethering has a number of drawbacks. First, the conjugation chemistry for tethering can be challenging. Second, the spatial confinement and the nonspecific attraction from the surface can induce artifacts to the dynamics of the molecule. For semi-rigid objects, such as actin filaments, surface attachment could also induce bending and twisting of the filament, complicating the applied force profile. To overcome these issues, we present a surface-free force spectroscopy method that uses a high-speed cross-slot hydrodynamic trap, capable of stretching molecules and cells with hydrodynamic drag. The trap is based on a glass microfluidic cross-slot flow chamber. Buffer flows in from two opposite directions and exits via the two orthogonal outlets to create an elongational flow field with a stagnation point in the center. As a result, objects near the stagnation point are stretched by the viscous drag from the flow. In addition, the pressure in one of the outlet reservoirs is electronically controlled with a high-speed feedback algorithm to stabilize the object at the stagnation point. Thanks to the high-speed feedback, we can apply much higher flow rate and therefore much higher stretching force on the trapped object than with the cross slot alone. We demonstrate tension-dependent actin severing, extension of von Willebrand Factor (a key protein in haemostasis that changes conformation in response to hydrodynamic stress in blood stream), overstretching of double strand DNA, and stretching deformation of red blood cells. In summary, the high-speed cross-slot hydrodynamic trap can be a powerful, surface-free alternative to more commonly used force spectroscopy methods.

1786-Plat
High-Speed Force Spectroscopy Unbinds Streptavidin-Biotin at the Velocity of Molecular Dynamics Simulations
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The forced disruption of the (strept)avidin-biotin complex by atomic force microscopy (AFM) and other techniques opened the field and established the basis of single molecule force spectroscopy (1-3). Steered molecular dynamics (SMD) simulations provided atomic description of the unbinding process (4). However, the maximum experimental AFM velocities were typically of 10 μm/s, while SMD simulations were performed in the range of 1 m/s to 10 m/s. Recent development of high-speed force spectroscopy (HS-FS) using HS-AFM allowed velocities in the mm/s range (5). We have applied HS-FS to probe the binding strength of the streptavidin-biotin complex at velocities up to ~8 mm/s, paralleled by SMD simulations. The experimental dynamic force spectrum of the unbinding process is compared with SMD simulations. The combination of HS-FS and SMD at overlapping velocities provides an atomic description of the unbinding process measured by experiment.
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The Princess and the Pea: A Story of Cell Mechanics
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Single molecules of integrins, a class of trans-membrane proteins involved in cell adhesion, experience a peak force of 40 pN through their ligands during initial adhesion. This force requirement was determined using a series of double stranded (ds) DNA tethers, each with a different rupture force, conjugated to the ligand. Here, force spectroscopy is performed by multiplexing two types of DNA tethers on the same surface: one that requires a strong rupture force (54 pN) and the other, a weak rupture force (12 pN). When presented alone, cells adhere to the strong tethers but not the weak tethers. However, when multiplexed, the result is synergistic; cells can adhere to a surface displaying just a few molecules of the strong tether if, and only if, they are presented along with many weak tethers. This degree of ultra-sensitivity raises a question on how cells suppress noise during decision making based on nano-mechanical environments.

Platform: Protein-Small Molecule Interactions

1788-Plat
Biasing Potential Replica Exchange Multi-Site λ-Dynamics for Efficient Free Energy Calculations of Protein-Ligand Interactions
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Traditional free energy calculations are known for their issues with scalability, speed and convergence. We have recently developed the biasing potential replica exchange multi-site λ-dynamics (BP-REX MSλD) method, which is a free energy method capable of transforming multiple substituents at multiple sites on a common framework with relative ease. With this methodology, we are able to show convergence of flexible moieties for symmetric benzoquinone derivatives, and have developed a series of metrics capable of increasing the λ-space sampling. We grouped and ordered the substituents based on volume occupancy, and were able to compute the free energy of binding for a series of challenging geldanamycin-derivatives for heat shock protein 90. The perturbations spanned by as much as 60 $Å^3$ and we were able to model these with a 2.4 kcal/mol average unsigned error. These metrics coupled with BP-REX MSλD allow for routine calculations on the order of hundreds of compounds in a few simulations.

1789-Plat
Survey of Phosphorylation Near Drug Binding Sites in the Protein Data Bank (PDB) and their Effects
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While it is currently estimated that 40-50% of eukaryotic proteins are phosphorylated, little is known about the frequency and local effects of phosphorylation near pharmaceutical inhibitor binding sites. In this study, we investigated how frequently phosphorylation may affect the binding of drug inhibitors to target proteins. We examined the 453 non-redundant structures of soluble mammalian drug target proteins bound to inhibitors currently available in the Protein Data Bank (PDB). We cross-referenced these structures with phosphorylation data available from the PhosphoSitePlus database. 322/453 (71%) of drug targets have evidence of phosphorylation that has been validated by multiple methods or labs. For 132/453 (29%) of those, the phosphorylation site is within 12Å of the small molecule-binding site, where it would likely alter small molecule binding affinity. We propose a framework for distinguishing between drug-phosphorylation site interactions that are likely to alter the efficacy of drugs vs. those that are not. In addition we highlight examples of well-established drug targets, such as estrogen receptor alpha, for which phosphorylation may affect drug affinity and clinical efficacy. Our data suggest that phosphorylation may affect drug binding and efficacy for a significant fraction of drug target proteins.

1790-Plat
Identification and Characterization of Protein-Protein Interaction Effectors Targeting the Invasion Machinery of the Malaria Parasite
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Emerging resistance of *Plasmodium falciparum* to current treatments is concerning and requires the development of a new generation of drugs. In pursuit of novel compounds, we have targeted protein-protein interactions (PPIs) essential to the parasite.
Plasmodium uses an actomyosin motor, part of the glideosome complex, for gliding and invasion of host cells. A key interaction of the glideosome is between aldolase and the cytoplasmic tail of the thrombospondin-related adhesive protein, TRAP. It is thought that the dynamic aldolase-TRAP complex must dissociate for forward motility and invasion to progress; therefore, we have developed an assay to identify compounds that promote this interaction, resulting in stalled gliding and invasion.